

Inventor(s): Elbaz et al. Application No: 09/762,194 Notice of Allowance Dated July 15 2004 Atty Dkt No: 033339/208804

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LOCUS

AT2 receptor C-terminal end

160 BP DS-DNA

ORGANISM BASES Mouse 41 A

33 · C

36 G

50 T

Nucleic acids

1 TGTGTTAATC CCTTCCTGTA TTGTTTTGTT GGAAACCGCT TCCAACAGAA CGTCCGCAGT GTGTTTAGAG TTCCCATTAC TTGGCTCCAA GGCAAGAGAG AGACTATGTC TTGCAGAAAA

121 GGCAGTTCTC TTAGAGAAAT GGACACCTTT GTGTCTTAAA

### Translation into amino acids

CVNPFLYCFV GNRFQQNVRS VFRVPITWLQ GKRETMSCRK GSSLREMDTFVS.

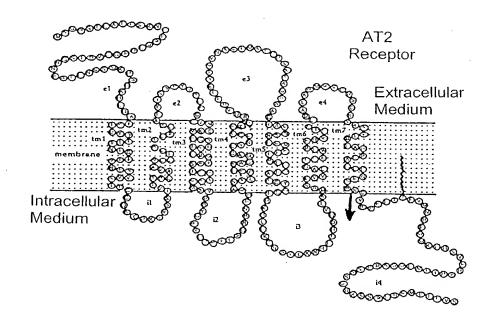


Figure 1



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Codon 147 .... AGT AAC AAA GGT CAA AGA CAG TTG ACT GTA TCG

GAL4 DNA-binding domain

Smal

Multiple Cloning Site

Sall

CCG GAA TTC CCG GGG ATC CGT CGA CCT...

BamHI

EcoRI

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	GCTA	CTACCCCCCCCACGCACCCCCAATCTGGGTGGCCTGGCATTAGCATGTAAGCTTGTTTTTCTCTGGC									71								
	TGTA	ATCTO	CTTGC	CCTC	GAAC	GAACO	CCGA	GTTC	CCA	AGAGA	ACACA	AGTAT	GTGA	TGGT	CCCI	GGAA	AAGC	TGCT	143
	TCCC	CCTGC	CGAAC	TTCI	CCCA	ACTGO	SCTTC	GAAG	GAC A		L CTG T	L TG I	S CT C	P CCC F	K AAA 1	F TC T	s CCC I	L TA	9 204
	S TCC	T ACC	I ATC	H CAC	V GTC	R CGC	L CTA	T ACC	A GCC	K AAA	G GGA	L CTG	L CTT	R CGA	N AAC	L CTC	R CGG	L CTT	27 258
	PCCT	S TCG	G GGG	L CTC	R AGG	K AAA	N AAC	T ACT	V GTC	I ATT	F TTC	H CAC	T ACA	V GTT	E GAA	K AAG	G GGC	R AGG	45 312
	Q CAG	K AAG	N AAT	P CCC	R AGG	S AGC	L CTG	C TGC	I ATC	Q CAG	T ACC	Q CAG	T ACA	A GCT	P CCA	D GAT	V GTG	L CTG	63 366
	S TCC	S TCC	E GAG	R AGA	T ACG	L CTT	E GAG	L TTG	A GCC			K AAG			C TGT	E GAA	S AGC	Q CAA	81 420
	S AGT	G GGA	F TTC	I ATC	L CTG	H CAC	L CTC	R AGG	Q CAG	L CTT	L CTT	S TCC	R CGT	G GGT	N AAC	N AAC	K AAG	F TTT	99 474
1	E GAA	A GCG	L CTG	T ACA	V GTT	V GTG	I ATC	Q CAG	H CAC	L CTC	L CTG	S TCT	E GAG	R CGG	E GAG	E GAA	A GCA	L CTG	117 528
	K AAG	Q CAA	H CAC	K AAA	T ACC	L CTC	S TCT	Q CAA	E GAA	L CTT	V GTC	S AGC	L CTC	R CGG	G GGA	E GAG	L CTA	V GTT	135 582
	A GCT	A GCT	S TCA	S AGC	A GCC	C TGT	E GAG	K AAG	L CTA	E GAA	K AAG	A GCT	R AGG		D GAC	L TTA	Q CAG	T ACA	153 636
	A GCG	Y TAT	Q CAA	E GAA	F TTT	V GTC	Q CAG	K AAA	L CTA	N AAC	Q CAG	Q CAG	H	Q CAG	T ACA	D GAC	R CGG	T ACG	171 690
	E GAA	L CTG	E GAG	N AAC	R CGG	L CTG	K AAG	D GAC	L TTA	Y TAC	T ACC	A GCA	E GAG	C TGT	E GAG	K AAG	L CTT	Q CAG	189 744
	S AGC	I ATT	Y TAC	I ATT	E GAG	E GAĠ	A GCA	E GAA	K AAA	Y TAT		T ACT	Q CAA	L CTG	Q CAA	E GAG	Q CAG	F TTT	207 798
رکه	D GAC	N AAC	L TTA	N AAC	A GCC	A GCC	H CAT	E GAG	T ACC	T ACT	K AAG	L CTT	E GAG	I ATT	E GAA	A GCT	S AGC	H CAC	225 852
	S TCG	E GAG	K AAG	V GTG	E GAA	L TTG	L CTG	K AAG	K AAG	T ACC	Y TAT	E GAA	T ACC	S TCC	L CTT	S TCA	E GAA	I ATC	243 906
3	K AAG	K AAG	S AGC	H CAT	E GAG	M ATG	E GAG	K AAG	K AAG	S TCA		E GAG	D GAT	L CTG	L CTT	N AAT	E GAG	K AAG	261 960
	Q CAG	E GAA	S TCG	L CTG	E GAG	K AAA	Q CAA	I ATC	N AAT	D GAT	L CTG	K AAG	S AGT	E GAA	N AAC	D GAT	A GCT	L TTA	279 1014
	N AAC	E GAA	R AGG	L TTG	K AAA	S TCA	E GAG	E GAG	Q CAA	K AAG	Q CAA	L CTG	S TCA	R AGA	E GAG	K AAG	A GCG	N AAT	297 1068
	S TCC	K AAA	N AAC	P CCT	Q CAG	V GTC	M ATG	Y TAT	L CTG	E GAG	Q CAA	E GAA	L CTA	E GAA	S AGC	L CTG	K AAG	A GCT	315 1122

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V GTG	L TTA	E GAG	I ATC	K AAG			K AAG			~	~	D GAC	M ATG	K AAG	L CTA	M ATG	K AAG	333 1176
M ATG	E GAA	K AAG	L CTG		D GAC							D GAC	K AAG	L CTG	K AAG	R CGA	F TTC	351 1230
Q CAG	Q CAG	E GAA	N AAC	E GAG	E GAG	L TTA	K AAA	A GCT	R CGC	M ATG	D GAC	K AAA	H CAC	M ATG	A GCA	I ATT	S TCA	369 1284
R AGG	Q CAA	L CTT	S TCC	TACC	E GAG	-	A GCC			_	E GAG	S TCC	L CTT	E GAG	K AAG	E GAG	S TCA	387 1338
K AAG	V GTC	N AAC	K AAG	R AGA	L CTG	S TCC	M ATG	E GAG			E GAA	L CTT	L CTG	W TGG	K AAA	L CTG	H CAC	405 1392
N AAC	G GGA	D GAC	L CTG		S AGC							S TCC	S TCG	A GCC	I ATC	P CCT	F TTC	423 1446
Q CAG	S TCC	P CCC	R AGG		S TCT								I ATC	_	CCC	R AGA	* TGA	440 1500
CGG	CTTC	rgaa	CGCA	GGAG.	ACTC'	rctg.	AAGG	CACT	GAGG	TGCG	CTTC	TGCA	GGAC'	TGAC	CCTC	TCAT	GGGA	1571
ACT	CGAG'	TTGC'	TGCG	TTAG	CTCT	CTGG.	AATA'	rccc	CAGG.	ATAT	CGGG	AGAG	CAGC	CGCC.	AACC	GTAT	CAGC	1642
TAC	GTAC	GAAT.	AGAG.	AGCT	CCAA	TAGA.	AGAC'	TTTT.	AACT	TGGT	CCAA	AAGC	CTCC	TCCA	AAAA	CAGA	TTTC	1713
GGAACTGAAGTGGACATAGTTGCACAAAGCACTTACGGAACGAGGGAACCTTGTTCTTTGCCTTCCTT										1784								
CTAAGCATAGGCTTTCCAG										1803								

### Figure 3.2

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### Title: NUCLEIC SEQUENCES CODING FOR AN AT2 INTERACTING PROTEIN INTERACTING WITH THE AT2 RECEPTOR ...

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72 144 cctctgtggtggaatgacatttgctgtgtaggcatctttcctctgactgtatttcttggccttgaagagtac 216 tgagtttaaaaagacagtatgtgacagtccatggaaattgcctcttctgtgaaatctcgccacctgctccga 288 agac ATG TTG TCT CCC AAA TTC TCC TTA TCC ACC ATT CAC ATA CGA CTG ACG 343 17 K F S. L S T Ι Н Ι R L L S P GCC AAA GGA TTG CTT CGA AAC CTT CGA CTT CCT TCA GGG TTT AGG AGA AGC ACT 397 P S т 35 R S G F R L L R Ν G GTT GTT TTC CAC ACA GTT GAA AAG AGC AGG CAA AAG AAT CCT CGA AGC TTA TGT 451 S R K N ₽ R S  $\mathbf{L}$ C 53 F Н E K Q V 505 GAT GCG CTG CCC CCT GAG AAA ACA CTT GAA TTG ATC CAG CCA CAG ACA GCT CCC 71 P D Α L P Ρ E K Т Τ. E Τ, Ι Q T ATC CTG CAG CTC AAG 559 CAA AGT GGA TTT ACG CAA TAT AAA ACA AAA TGT GAA AAC S F Ι L 0 L K 89 Ē N Q G Y K T K C ACA GTT GCC TGT GGT AAT ACC AAG TTTGAG GCA TTG GTG ATT CAG 613 CTTCAG CTT Ι Q 107 G N т K E Α Т v V Τ, Α  $\mathbf{C}$ Q L 667 AAA ACC CTA TCT CAA CAC CTG CTG TCT GAG CGG GAG GAA GCA CTG AAA CAA CAC 125 K K ጥ L S Q Η L S Ε R E E O 721 ✓ GAA GTC ACT GCT TCA ACC ACC TGTGAG AAA GTT AAC CTC CGG GGA GAG CTA CTT 143 E K G Ε L V Т A S Т ጥ C E L N R 775 AAT GAG TTA CAA TAT GAA GCA TTC GTC CAG CAG GCC AGG ACA GTG TTAGAA AAA E F 161 T Y A E Κ Α R ٠N Ē L Q L 829 GAG TTT TAC ACC AGG ACA GAA CGA GAG AATCGG CTTAAA CAC CAG GCT GAA AAA 179 K F Y Т R E E Ε Ē K Q Α ACT TAC ATT GAA GAA GCA GAG AAG TAC AAA ATG 883 GAG TATGAA AAG CTT CGG GAC 197 K M D т Y Т F. F Α E K Y E R 937 TAA CAT GAA ACC TCT AAG TTG GAA CAA TTG CAA GAG CAG TTTGAC AAC TTA GCG 215  $\mathbf{T}$ S K Ε N Α Н Ε Q L 0 E Q F D N L GCC TAT GAA GCC 991 AAA CTT GAA TTG CTA AAG AAG TCA GAG ATT GAA GCT AGC CAC 233 K Ë Α E L L E K L Ε Α S Η S Ι 1045 GAA ATA GAA AAG AAA TCG CTT GAA GAT GAA ATT AAG AAA GGC CAT CTT TCA TCC 251 G Н Ē K S L E D E Т K K S L S 1099 CTG AAG AGT TCG CTA GAG AAG CAA ATC AAT GAT ATT CTT TCT GAG AAG CAG GAA 269 K S Ε S E K O Ι N D L S E K Q L AGA AGA GCA 1153 CAA AAAAAA TTG AAATCA GAA GAA AAT GAT GCT TTA TAAGAA GAA 287 K R R Α Ε K L K S Ε E Q Ε N D Α N L CTA GAA CAG GAG TTA 1207 AAT CCT CAG ATC ATG TAT AGA GAA AAA GCA AAT TTGAAA F. L 305 E Q K N Ρ Q I М Y L R E K Α

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GAA AGC CTG AAA GCT GTG TTA GAG ATC AAG AAT GAG AAA CTG CAT CAA CAG GAC 1261 323 E Ι К Ν K Κ ATC AAG TTA ATG AAA ATG GAG AAA CTG GTG GAC AAC AAC ACA GCA TTG GTT GAC 1315 341 Ε K L V D N N Μ AAA TTG AAG CGT TTC CAG CAG GAG AAT GAA GAA TTG AAA GCT CGG ATG GAC AAG 1369 359 Ε N Ε Ε ĸ R M D CAC ATG GCA ATC TCA AGG CAG CTT TCC ACG GAG CAG GCT GTT CTG CAA GAG TCG 1423 377 T S Ε Α R CTG GAG AAG GAG TCG AAA GTC AAC AAG CGA CTC TCT ATG GAA AAC GAG GAG CTT 1477 395 E E S Ν K V N К R L М CTG TGG AAA CTG CAC AAT GGG GAC CTG TGT AGC CCC AAG AGA TCC CCC ACA TCC 1531 413  $\mathbf{C}$ S K N G D L Н TCC GCC ATC CCT TTG CAG TCA CCA AGG AAT TCG GGC TCC TTC CCT AGC CCC AGC 1585 431 S G S S R Ν P Ι L 0 S ATT TCA CCC AGA TGA cacgtccccaaagtccacagactctctgaaagcattttgatgcaggtctgc 1651 aggactgaccccaaggaggaacgtgggcacaagaggtatatcagcacacgtgtgatcaccgtaggtaactgg 1723 agcgtcaccaccggcggaatcgagcttctgagactggaagtctggaggaagacttttgcctccgtccaaaag 1795 attcctccaaaaaaagatttaaaaaaagatttcggcatcgacacggacgttgttgcacaaagcacttaaaga 1867 acgagagcatcttgttcattgcctttttcacctaagcataaggggaaaaactctcagggccctattaagatt 1939 tataacctttgtaatgttcttcaccacagacaccttcttgtgagttttcagtctgactgtgggggtgggggg 2011 tgtgaatgaaatggatgtcacagagtgtcatgtgtctgatgcagcctcctctgctgtgtattaaatgtcaaa 2083 atagaagaaaaagcaatagttgcttgaattatgatcatctaccaccaactctgctcagccctgtaacagggt 2227 agggagagggtataacaggaagagetttgacttgtccctgtctatacattctctgtatcttttgggggtaac 2299 tgtgagcctaacactatcctgtaattcattttctcaggctatgtgtaaatgtagaaccctaatttttctata 2443 aaaaaacaaactaactaactgtgtaaagaaagaaaaagggaagtaccaatgggtttttccaccttatttta 2515 atgattttccagagccccagagccagctcatcttccaggtgctgaaaccactttccaaataaactaaagcct 2731 ggatttgatattacaaattttgggaaatcttagaataaagaacgagaacaaggaagtcattggctagtataa 2803 ttaagaaaggtaggattcagtgcttaccgatgatgcagtacttgatagaagaaaacagtctgggaggatagc 2875 gctcatttttcagttaccctttaaggagtccctttgtctttgggaaagtagcagaatggtccgcttctttcc 2947 

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cccctaatcctgagactttggaaaaggtggaaggaagaactgttgctttatctccccctccct	3091
caacattgtgatgtcagtatttactaatctacattcagtggctgtacaaataacagctgtagtaagaagaga	3163
tcaggatgctagaggtgaatatttgggtcatttacatgtacactacatagcaagttgatactcatgttgca	3235
gttcttttaaattagtgattttgtgtcttaagtctttaacttccaatacttcatcatgtatgt	3307
atgtttgcttctgataaatggaaatgtaggttcactgccacttcatgagatatctctgctcacgcttccaag	3379
tgttctcaatgacattagccaaagttgggtttgccattcatcccctaggcatggtaaatcttgtgtttc	3451
cctgctgtcctccgtattacgtgaccggcaaataaatctcatagcagttaatataaaacatctttggaggat	3523
ggagagaacaggagggaagatgggaaacaaaatagagaattcttaagattttgtttaaaccaaatgtttca	3595
gtagaatgcaaaatgttggcacgtcaaaaatatgaatgtgtagacaactgtagttgtgctcagtttgtagt	3667
gatgggaagtgtattttactctgatcaaataaataatgctggaatactcaaaaaaaa	3739
aaa	3742

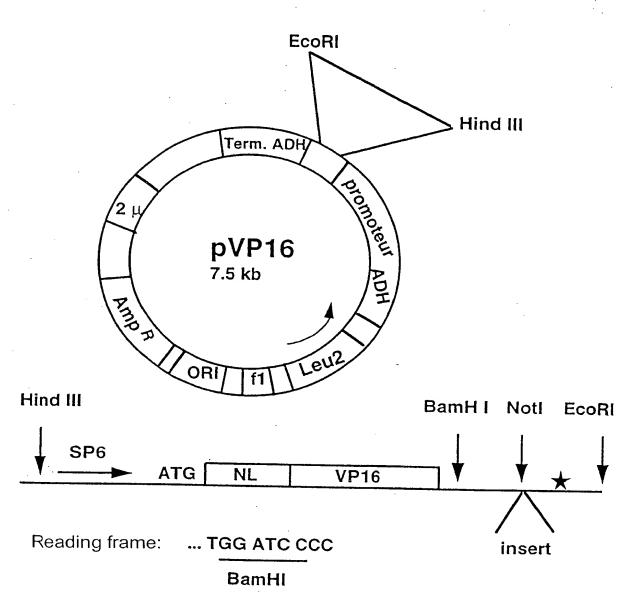
### Figure 4.3



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★ Stop codons in three frames
pVP16 was constructed by Stan Hollenberg

Figure 5

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GAT	TCC H-	CGA	AAG	•
CGG	GGA Ban	ATT	AGG	TAG
GGT	TGG	GGA	GAA	AAC
ATG	CGA	CAT	CCC	CTG AGT TGG CTG CCG CTG AGC AAT AAC TAG
CAA	GAT	TAC	AAG	AGC
CAG	AAG	TGG	ACA	CTG
GGA	GAT	AGC	CTA	೮೦೦
GGT	GAC	TGC	CTG	CCA
ACT	GAT	ATC	. යයය	CTG
ATG	GAC	GAG	ATC	TGG
AGC	TAC	CIC	TTG	AGT
GCT	CTG	GAG	AGC	
134	170	206	242	278
	134 GCT AGC ATG ACT GGT GGA CAG CAA ATG GGT CGG GAT			

6 histidines

Figure 6

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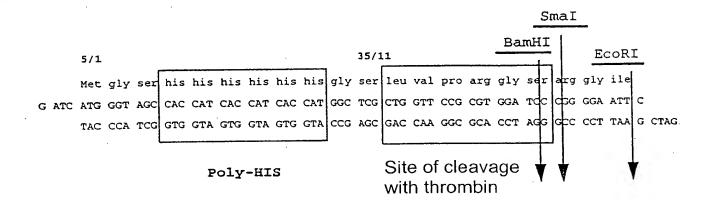
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TAC CCA, GGC CTT GTC TTT GAC TAG AGA CTT CTT CTG GAC, CCT AGG CCT TAA GAT CT ATG GGT CCG GAA CAG AAA CTG ATC TCT GAA GAA GAC CTG'GGA TCC'GGA ATT CTA GA Met gly pro glu gln lys leu ile ser glu glu asp leu gly ser gly ile leu Bar Tag Myc

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pBacPAKI-poly HIS -> Graphic Map

5526 b.p. AACGGCTCCGCC ... TCATTAATGCAG circular

PolyHIS insertion into pBackpack in BamHI (CACCAT) 3 1270-1287

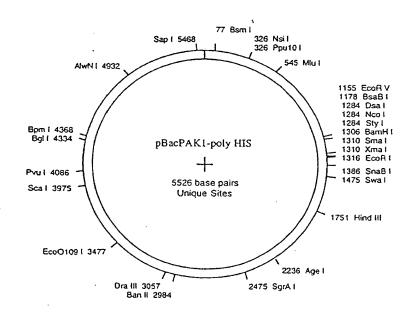


Figure 8

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Tissues:

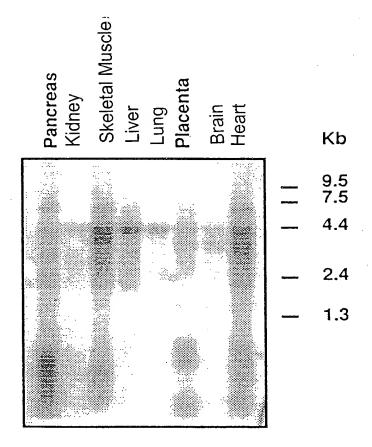


Figure 9



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			✓ MBP-AT2	← GST-ATIP ← GSTalone		
nts:	MBP-AT2 MBPv MBP-AT1				I +	+
Supernatants:	MBPv				i +	+ i
Sn	WBP-AT2				1+	+
	I	KDa	48 –	33	GST-ATIP	GSTalone
		Antibodies	anti-MBP	anti-GST	noode.	

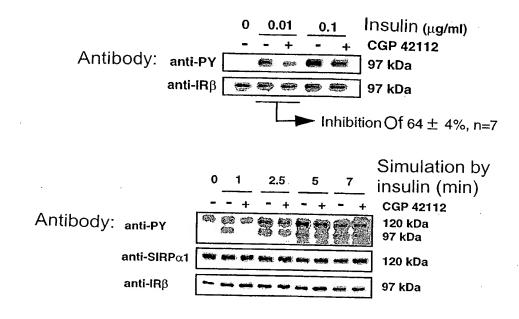


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### CHO-hAT2

### Lectin column



### CHO-hAT2 et CHO-hAT2-ATIP

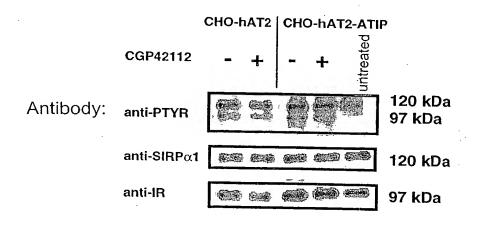


Figure 11